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Cutting Edge: Selective Up-Regulation of Chemokine Receptors CCR4 and CCR8 upon Activation of Polarized Human Type 2 Th Cells[1]

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Polarized Th1 and Th2 cells differentially express adhesion molecules and chemokine receptors, endowing these cells with distinct tissue homing capabilities. Here we report that, in contrast to other chemokine receptors, the expression of CCR4 and CCR8 on Th2 cells is transiently increased following TCR and CD28 engagement. IL-4 is not required for this activation-induced up-regulation of CCR4 and CCR8. In accordance with receptor expression, the response of Th2 cells to I-309 (CCR8 ligand) and thymus- and activation-regulated chemokine (CCR4 and CCR8 ligand) is enhanced upon activation. Moreover, activated Th1 cells up-regulate CCR4 expression and functional responsiveness to thymus- and activation-regulated chemokine. Analysis of polarized subsets of CD8$^+$ T cells reveals a similar pattern of chemokine receptor expression and modulation of responsiveness. Taken together, these findings suggest that an up-regulation of CCR4 and CCR8 following Ag encounter may contribute to the proper positioning of activated T cells within sites of antigenic challenge and/or specialized areas of lymphoid tissues. ***The Journal of Immunology*****, 1998, 161: 5111–5115.**

The differentiation process of CD4$^+$ T cells proceeds along two distinct pathways leading to the generation of polarized effector Th cells (1–3). IFN-γ-producing Th1 cells promote phagocyte-dependent immunity (4, 5). Th2 cells secreting IL-4 and IL-5 promote IgE production and allergic responses (6). Similar to the generation of CD4$^+$ Th1 and Th2 cells, naive CD8$^+$ T cells can differentiate into Th1 type 1 cytotoxic CD8$^+$ T cells (Tc1)[3] cells or Tc2 type cytotoxic CD8$^+$ T cells (7).

A multistep process mediated by the interplay of adhesion molecules and chemokines that involves rolling, firm adhesion, and diapedesis results in the extravasation of immune effector cells within peripheral tissues (8, 9). Recent findings indicate that P- and E-selectin ligands (10) and chemokine receptors (11, 12) are differentially expressed on Th1 and Th2 cells, providing these cells with distinct tissue-homing abilities (13). Chemokines are members of a large family of small cytokines that play a key role in the leukocyte-recruitment process (14–16). We and others have reported recently that among eight CC and four CXC chemokine receptors, Th1 cells predominantly express CXCR3 and CCR5 (12, 17, 18); Th2 cells selectively express CCR3 (11), CCR4 (12, 17), and CCR8 (19). Although these findings suggest that different chemotactic signals are required for extravasation, migration, and tissue homing, the hierarchy and composition of these signals are unknown and may depend upon the chemokines present in the tissue and the chemokine receptors expressed on the invading cells.

Here, we investigated the effect of Ag receptor triggering on the expression pattern of chemokine receptors on Th1 and Th2 cells. Our data indicate that, in contrast to other chemokine receptors, the expression of CCR4 and CCR8 on Th2 cells and CCR4 on Th1 cells is markedly increased upon activation. These findings suggest a unique role for these receptors in extravasation, tissue migration, and the positioning of effector T cells.

Materials and Methods

Type 1 and type 2 lines and Th2 cell clones

Type 1 and type 2 cell lines were generated from cord blood lymphocytes and maintained in culture as described previously (20). CD4$^+$ Th1 and Th2 cells and CD8$^+$ Tc1 and Tc2 cells were purified by immunomagnetic negative selection using anti-CD4 or anti-CD8 mAb-coated microbeads according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The Lolium perenne group-1-specific Th2 clone D4.11, which was obtained as described previously (12), was restimulated with PHA and irradiated PBMCs and cultured in complete medium with IL-2.

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[3] Abbreviations used in this paper: Tc, cytotoxic CD8$^+$ T cell; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; AM, acetoxymethyl ester; IP-10, IFN-inducible protein-10; TARC, thymus and activation-regulated chemokine.

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Northern blot analysis

For Northern blots, total RNA was extracted from polarized $CD4^+$ or $CD8^+$ T cells by TRIzol (Life Technologies, Grand Island, NY). For activation experiments, purified $CD4^+$ Th1 and Th2 cells were either left untreated or incubated for 16–24 h on anti-CD3 (Tr66 mAb)-coated plates with 1 μg/ml of anti-CD28 mAb (PharMingen, San Diego, CA). The Th2 clone D4.11 was either left untreated or incubated for 24 h on anti-CD3-coated plates with 1 μg/ml of anti-CD28 mAb. TCR triggering was terminated by washing and culturing the cells in complete medium with IL-2. Untreated and TCR-stimulated cells were lysed at various times after the termination of TCR-triggering. Total RNA was extracted, and equal amounts of RNA (10 μg/lane) were fractionated on a 1% agarose-formaldehyde gel. The specific mRNAs were detected by the hybridization of nylon membranes (NorthernMax Kit, Ambion, Austin, TX) with ^{32}P-labeled DNA probes for human CCR3, CCR4, CCR5, CCR8, and CXCR3 according to the manufacturer's instructions (NorthernMax Kit, Ambion). The filters were exposed to X-OMAT AR film (Eastman Kodak, Rochester, NY) between double intensifying screens (DuPont, San Diego, CA) at −70°C. The extent of the hybridization was quantified by densitometric analysis with the entry level image system (Immagini e computer, Milan, Italy), and the values were normalized using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal as a reference.

Analysis of intracellular calcium mobilization

Type 1 and type 2 polarized T cells were either left untreated or activated as described above. Next, cells were washed and rested by incubation in complete medium with IL-2 for 12 h. Fluo-3/acetoxymethyl ester (AM) loading (21) was performed by incubating the cells (5×10^6/ml) in buffer A (HBSS with 10 mM HEPES) with 2 μM fluo-3/AM (Molecular Probes, Eugene, OR) at 37°C for 30 min. The incubation was prolonged by 30 min after the addition of an equal volume of buffer B (HBSS with 10 mM HEPES and 5% FCS). Cells were washed twice in buffer B and stained with quantum red-conjugated anti-CD4 or anti-CD8 Abs (Sigma, St. Louis, MO). Cells were washed, resuspended at 2×10^6/ml, and analyzed by FACS. Emissions at 525 and 613 nm were measured on a log scale before and after stimulation with the chemokines (IFN-inducible protein-10 (IP-10), eotaxin, I-309, and thymus- and activation-regulated chemokine (TARC) were purchased from R&D Systems, Minneapolis, MN). Analysis was restricted to $CD4^+$ or $CD8^+$ T cells by gating on $FL3^+$ cells, with an acquisition of 3000 events.

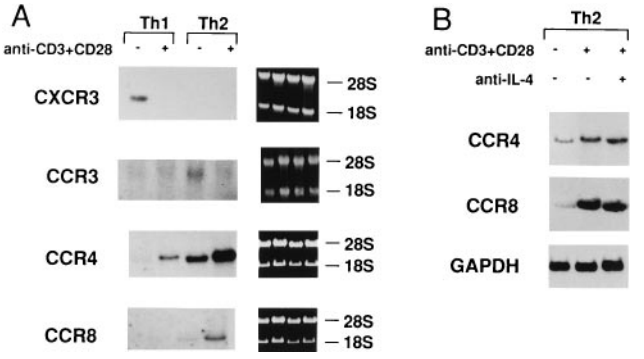

FIGURE 1. Activation-dependent regulation of chemokine receptor expression in Th1 and Th2 cells. *A*, Th1 and Th2 cells were either left untreated or cultured on anti-CD3 mAb-coated plates with soluble anti-CD28 mAb. Cells were harvested, and total RNA was extracted. A total of 10 μg of total RNA was subsequently used in Northern blot analysis. Filters were hybridized with ^{32}P-labeled probes for CXCR3, CCR3, CCR4, and CCR8. Equivalent loading of RNA was verified by ethidium bromide staining of 18S and 28S ribosomal RNA. *B*, Th2 cells were either left untreated or cultured on anti-CD3 mAb-coated plates with soluble anti-CD28 mAb in the presence or absence of anti-IL-4 mAb (500 ng/ml). A total of 10 μg of total RNA was subsequently used in Northern blot analysis. Filters were hybridized with ^{32}P-labeled probes for CCR4, CCR8, and GAPDH.

Results and Discussion

To study the regulation of chemokine receptor expression on Th cells, we generated T cell lines from leukocytes isolated from cord blood as described previously (20). The Th phenotype of $CD4^+$ and $CD8^+$ T cells was determined by restimulation and intracellular staining for IFN-γ and IL-4 production (data not shown). Purified $CD4^+$ Th1 or Th2 cells were either left in culture with

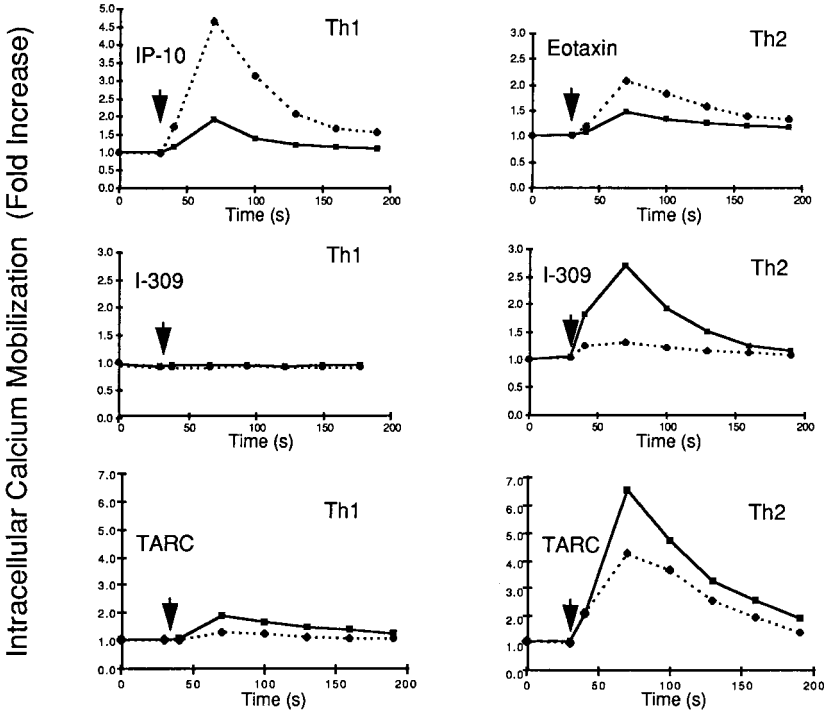

FIGURE 2. Chemokine-induced intracellular Ca^{2+} mobilization in resting and activated Th1 and Th2 cells. Th1 and Th2 cells were either left untreated or activated as described in Fig. 1. Cells that had been loaded with fluo-3/AM and stained with quantum red-conjugated anti-CD4 Abs were analyzed by flow cytometry by gating on $CD4^+$ cells. Resting (dashed lines) and activated (solid lines) Th1 and Th2 cells were stimulated with IP-10 (50 ng/ml), eotaxin (400 ng/ml), I-309 (200 ng/ml), and TARC (10 ng/ml). The time of the addition of the chemokines is indicated by the arrow. The response is expressed as the fold increase of mean fluorescence intensity at time 0 for emissions at 525 nm. One representative experiment of five is shown.

IL-2 or cultured on anti-CD3 mAb-coated plates with soluble anti-CD28 mAb. Subsequently, the cells were lysed, and total RNA was extracted and analyzed for CXCR3, CCR3, CCR4, and CCR8 mRNA expression. In agreement with recent reports (11, 12, 18, 19), CXCR3 is preferentially expressed on Th1 cells, whereas CCR3, CCR4, and CCR8 are preferentially expressed on Th2 cells (Fig. 1*A*). Stimulation with anti-CD3 and anti-CD28 Abs down-regulates the mRNA expression of CXCR3 in Th1 cells and CCR3 in Th2 cells (Fig. 1*A*). In contrast, CCR4 and, to a greater extent, CCR8 transcripts are up-regulated in TCR-stimulated Th2 cells (Fig. 1*A*). In addition, CCR4 mRNA becomes detectable in TCR-stimulated Th1 cells (Fig. 1*A*).

Since CCR4 and CCR8 are expressed on Th2 cells that have been generated in vitro in the presence of IL-4, it is possible that the IL-4 produced upon restimulation of Th2 cells is responsible for the increased expression of CCR4 and CCR8. Therefore, we performed the stimulation in the presence of anti-IL-4 blocking Abs. This experiment shows that blocking IL-4 does not inhibit the up-regulation of CCR4 and CCR8 upon activation of Th2 cells (Fig. 1*B*). Moreover, the addition of exogenous IL-4 or supernatants obtained from activated Th2 cells does not result in an up-regulation of CCR4 and/or CCR8 in Th1 cells or a further enhancement of these receptors in Th2 cells (data not shown).

To test the functional relevance of these observations, we explored chemokine responsiveness by measuring intracellular calcium mobilization following activation of Th1 and Th2 cells. Consistent with receptor expression, Th1 cells preferentially respond to IP-10 (CXCR3 ligand (22)), whereas Th2 cells selectively respond to eotaxin (CCR3 ligand (23)), I-309 (CCR8 ligand (24)), and TARC (CCR4 and CCR8 ligand (25, 26)) (Fig. 2). Stimulation with anti-CD3 and anti-CD28 Abs results in diminished responses of Th1 cells to IP-10 (Fig. 2, *upper left panel*) and of Th2 cells to eotaxin (Fig. 2, *upper right panel*). In contrast, the response to TARC and, more markedly, to I-309 is increased upon activation of Th2 cells (Fig. 2, *middle* and *lower right panels*), and the response to TARC becomes detectable in activated Th1 cells (Fig. 2, *lower left panel*).

Since the experiments described above were performed on polarized T cell lines obtained by a single cycle of stimulation, we asked whether a T cell clone that had been subjected to repeated cycles of stimulation would exhibit a similar pattern of CCR4 and CCR8 regulation. TCR and CD28 triggering of a Th2 clone results in a marked up-regulation of CCR4 and CCR8 (Fig. 3). In addition, following termination of TCR triggering, the expression of CCR4

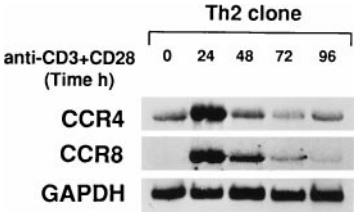

FIGURE 3. Transient up-regulation of CCR4 and CCR8 expression upon activation of a Th2 clone. The Th2 clone D4.11 was either left untreated or activated as described in Fig. 1. A fraction of the cells was lysed immediately (24 h). The remaining cells were washed, cultured, and harvested at 48, 72, and 96 h; total RNA was extracted. A total of 10 μg of total RNA was subsequently used for Northern blot analysis. Filters were hybridized with ^{32}P-labeled probes for CCR4, CCR8, and GAPDH.

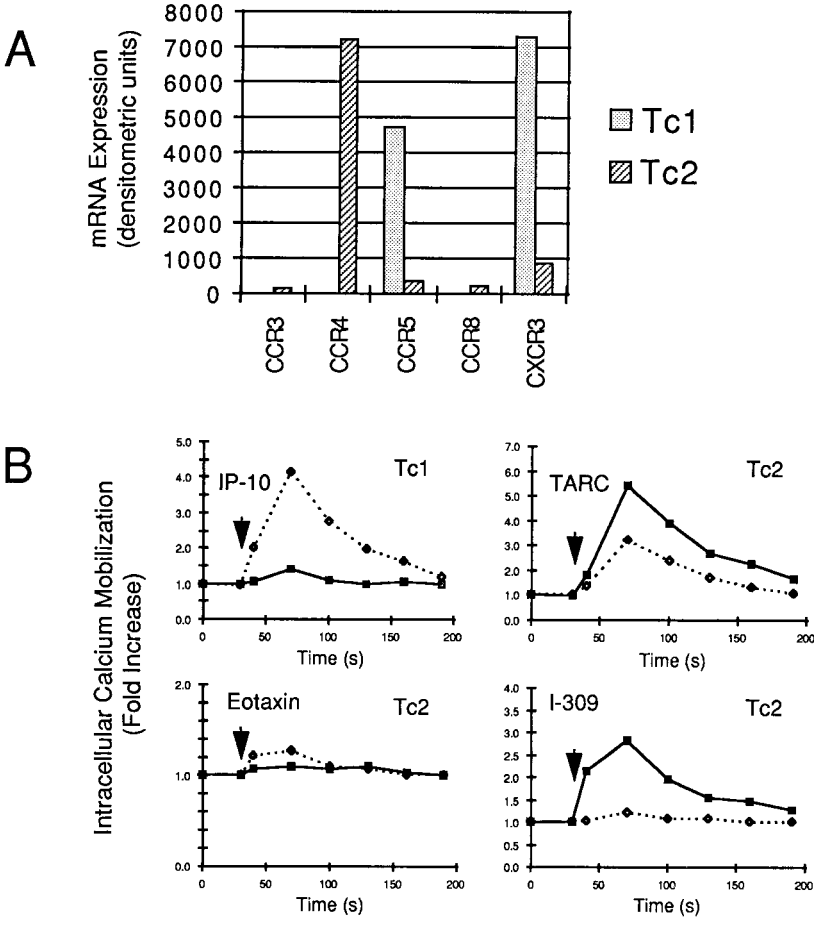

FIGURE 4. Chemokine receptor expression and modulation of chemokine responsiveness in Tc1 and Tc2 cells. *A*, A total of 10 μg of total RNA from Tc1 and Tc2 cells was used for Northern blot analysis. Filters were hybridized with ^{32}P-labeled probes for CXCR3, CCR3, CCR4, CCR5, and CCR8. A normalized densitometric analysis of a representative experiment is shown. *B*, Tc1 and Tc2 cells were either left untreated or activated, loaded with fluo-3/AM, stained with quantum red-conjugated anti-CD8 Abs, and analyzed by flow cytometry by gating on CD8$^+$ cells. Resting (dashed lines) and activated (solid lines) Tc1 and Tc2 cells were stimulated with IP-10 (50 ng/ml), eotaxin (400 ng/ml), I-309 (200 ng/ml), and TARC (10 ng/ml). The time of the addition of the chemokines is indicated by the arrow. The response is expressed as the fold increase of mean fluorescence intensity at time 0 for emissions at 525 nm. One representative experiment of three is shown.

and CCR8 returns to basal levels within 72 h (Fig. 3). Similar findings were observed with cord blood-derived Th2 cells (data not shown). These data indicate that Th2 cells transiently up-regulate CCR4 and CCR8 upon activation via the TCR. Collectively, our findings suggest that TCR-mediated activation of Th cells results in a transient alteration of the pattern of chemokine receptor expression.

Previous studies have demonstrated that, in addition to $CD4^+$ T cells, type 1 and type 2 populations of $CD8^+$ T cells (Tc1 and Tc2 cells) can also be generated in vitro and isolated in vivo in immunopathologic conditions (7, 27, 28). Thus, we have extended our analysis of chemokine receptors to polarized Tc1 and Tc2 cells generated from cord blood T cells. An analysis of the mRNA expression of several chemokine receptors revealed that, similar to $CD4^+$ Th cells, Tc1 cells preferentially express CXCR3 and CCR5 (Fig. 4*A*); Tc2 cells selectively express CCR3, CCR4, and CCR8 (Fig. 4*A*). To verify the modulation of chemokine responsiveness upon activation of polarized $CD8^+$ T cells, TCR- and CD28-stimulated Tc1 and Tc2 cells were assayed for their ability to mobilize calcium in response to various chemokines. The overall pattern of calcium mobilization in response to chemokines is concordant with the expression profile of chemokine receptors in Tc1 and Tc2 cells and is similar to Th1 and Th2 cells (Fig. 4*B* and data not shown). The response of Tc1 cells to IP-10 is greatly reduced following TCR-mediated activation (Fig. 4*B*). Activation of Tc2 cells results in a diminished response to eotaxin, whereas the response to TARC and I-309 is increased (Fig. 4*B*). Taken together, these data indicate that the pattern and regulation of chemokine receptor expression of polarized subsets of $CD8^+$ T cells may overlap that of $CD4^+$ T cells.

Recent studies have reported that TCR-mediated activation of T cells results in a down-modulation of CCR1, CCR2, CCR3, CCR5, and CXCR3 expression (11, 18, 29, 30). Our study reveals that, in contrast to other receptors, CCR4 and, to a greater extent, CCR8 are up-regulated upon TCR-mediated activation of Th2 cells. CXCR4 and CCR7 have been reported to be up-regulated upon activation of T cells (31, 32). However, recent work established that TCR triggering results in a down-modulation of CXCR4 that is subsequently reinduced by the autocrine action of secreted IL-4 (33). Thus, CCR7 is the only other receptor to be up-regulated upon TCR triggering (31). Interestingly, the CCR7 ligands, secondary lymphoid-tissue chemokine, and EBI1 ligand chemokine have been proposed to control the initial entry of naive T cells and the subsequent encounter of activated T and B cells in secondary lymphoid tissues (34, 35). Notably, CCR7 is also up-regulated upon activation of both Th1 and Th2 cells, suggesting a potential contribution to their migration patterns (A.I. and D.D., unpublished observations). Given their mode of regulation, CCR4 and CCR8 may play a role similar to CCR7 in focusing activated T cells to certain areas of lymphoid tissues. However, in contrast to CCR7, CCR4 and particularly CCR8 are selectively expressed on polarized effector Th2 cells, suggesting a unique function in localizing and positioning activated Th2 cells. It is noteworthy that CCR4 and CCR8 share many common features. Their sequence is highly homologous (36, 37); they are both highly expressed on thymocytes, basophils, and Th2 cells (12, 19, 24, 36); and both are receptors for TARC (26). Collectively, these data suggest that CCR4 and CCR8 may play a critical role in localizing activated Th2 cells within sites of antigenic challenge and/or to specialized areas of lymphoid tissues.

Great attention has recently been placed on the possibility of distinguishing Th1 vs Th2 cells on the basis of the differential expression of chemokine receptors. Our findings suggest that CCR8, which is highly expressed upon TCR-mediated activation, may be a very useful and selective marker for the in situ identification of recently activated Th2 cells.

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References

1. Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine Th T cell clones: definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol. 136:2348.*
2. O'Garra, A., and K. Murphy. 1994. Role of cytokines in determining T-lymphocyte function. *Curr. Opin. Immunol. 6:458.*
3. Seder, R. A., and W. E. Paul. 1994. Acquisition of lymphokine-producing phenotype by $CD4^+$ T cells. *Annu. Rev. Immunol. 12:635.*
4. Romagnani, S. 1994. Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol. 12:227.*
5. Abbas, A. K., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper T lymphocytes. *Nature 383:787.*
6. Coffman, R. L., and T. von der Weid. 1997. Multiple pathways for the initiation of T helper 2 (Th2) responses. *J. Exp. Med. 185:373.*
7. Seder, R. A., J. L. Boulay, F. Finkelman, S. Barbier, S. Z. Ben-Sasson, G. G. Le Gros, and W. E. Paul. 1992. $CD8^+$ T cells can be primed in vitro to produce IL-4. *J. Immunol. 148:1652.*
8. Springer, T. 1990. Adhesion receptors of the immune system. *Nature 346:425.*
9. Butcher, E. C., and L. J. Picker. 1996. Lymphocyte homing and homeostasis. *Science 272:60.*
10. Austrup, F., D. Vestweber, E. Borges, M. Lohning, R. Brauer, U. Herz, H. Renz, R. Hallmann, A. Scheffold, A. Radbruch, and A. Hamann. 1997. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature 385:81.*
11. Sallusto, F., C. R. Mackay, and A. Lanzavecchia. 1997. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science 277:2005.*
12. Bonecchi, R., G. Bianchi, P. Panina-Bordignon, D. D'Ambrosio, R. Lang, A. Borsatti, S. Sozzani, P. Allavena, P. A. Gray, A. Mantovani, and F. Sinigaglia. 1998. Differential expression of chemokine receptors and chemotactic responsiveness of Th1 and Th2 cells. *J. Exp. Med. 187:129.*
13. Lichtman, A. H., and A. K. Abbas. 1997. T-cell subsets: recruiting the right kind of help. *Curr. Biol. 7:242.*
14. Mackay, C. R. 1996. Chemokine receptors and T cell chemotaxis. *J. Exp. Med. 184:799.*
15. Hedrick, J. A., and A. Zlotnik. 1996. Chemokines and lymphocyte biology. *Curr. Opin. Immunol. 8:343.*
16. Baggiolini, M., B. Dewald, and B. Moser. 1997. Human chemokines: an update. *Annu. Rev. Immunol. 15:675.*
17. Sallusto, F., D. Lenig, C. R. Mackay, and A. Lanzavecchia. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med. 187:875.*
18. Loetscher, P., M. Uguccioni, L. Bordoli, M. Baggiolini, and B. Moser. 1998. CCR5 is characteristic of Th1 lymphocytes. *Nature 391:344.*
19. Zingoni, A., H. Soto, J. H. Hedrick, A. Stoppacciaro, C. T. Storlazzi, F. Sinigaglia, D. D'Ambrosio, A. O'Garra, D. Robinson, M. Rocchi, A. Santoni, A. Zlotnik, and M. Napolitano. 1998. The chemokine receptor CCR8 is preferentially expressed in Th2 cells but not Th1 cells. *J. Immunol. 161:547.*
20. Rogge, L., L. Barberis-Maino, M. Biffi, N. Passini, D. H. Presky, U. Gubler, and F. Sinigaglia. 1997. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J. Exp. Med. 185:825.*
21. Kao, J. P. Y., A. T. Harootunian, and R. Y. Tsien. 1989. Photochemically generated cytosolic calcium pulses and their detection by Fluo-3. *J. Biol. Chem. 264:8179.*
22. Loetscher, M., B. Gerber, P. Loetscher, S. A. Jones, L. Piali, I. Clark-Lewis, M. Baggiolini, and B. Moser. 1996. Chemokine receptor specific for IP-10 and Mig: structure, function, and expression in activated T-lymphocytes. *J. Exp. Med. 184:963.*
23. Ponath, P. D., S. Qin, T. W. Post, J. Wang, L. Wu, N. P. Gerard, W. Newman, C. Gerard, and C. R. Mackay. 1996. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J. Exp. Med. 183: 2437.*
24. Tiffany, L. H., L. L. Lautens, J.-L. Gao, J. Pease, M. Locati, C. Combadiere, W. Modi, T. I. Bonner, and P. M. Murphy. 1997. Identification of CCR8: a human monocyte and thymus receptor for the CC chemokine I-309. *J. Exp. Med. 186:165.*
25. Imai, T., M. Baba, M. Nishimura, M. Kakizaki, S. Takagi, and O. Yoshie. 1997. The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. *J. Biol. Chem. 272:15036.*
26. Bernardini, G., W. Luini, G. Spinetti, M. Weiss, S. Menon, A. Zlotnik, A. Mantovani, A. Santoni, and M. Napolitano. 1998. Identification of the CC chemokines TARC and macrophage inflammatory protein-1β as novel functional ligands for the CCR8 receptor. *Eur. J. Immunol. 28:582.*
27. Sad, S., R. Marcotte, and T. R. Mosmann. 1995. Cytokine-induced differentiation of precursor mouse $CD8^+$ T cells into cytotoxic $CD8^+$ T cells secreting Th1 or Th2 cytokines. *Immunity 2:271.*

28. Maggi, E., M. G. Giudizi, R. Biagiotti, F. Annunziato, R. Manetti, M. P. Piccinni, P. Parronchi, S. Sampognaro, L. Giannarini, G. Zuccati, and S. Romagnani. 1994. Th2-like $CD8^+$ T cells showing B cell helper function and reduced cytolytic activity in human immunodeficiency virus type 1 infection. *J. Exp. Med. 180:275.*
29. Loetscher, P., M. Seitz, M. Baggiolini, and B. Moser. 1996. Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. *J. Exp. Med. 184:569.*
30. Shixin, Q., J. B. Rottman, P. Myers, N. Kassam, M. Weinblatt, M. Loetscher, A. E. Koch, B. Moser, and C. R. Mackay. 1998. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J. Clin. Invest. 101:746.*
31. Burgstahler, R., B. Kempkes, K. Steube, and M. Lipp. 1997. Expression of the chemokine receptor BLR2/EBI1 is specifically transactivated by Epstein-Barr virus nuclear antigen 2. *Biochem. Biophys. Res. Commun. 215:737.*
32. Bleul, C. C., L. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay. 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc. Natl. Acad. Sci. USA 94:1925.*
33. Jourdan, P., C. Abbal, N. Nora, T. Hori, T. Uchiyama, J.-P. Vendrell, J. Bousquet, N. Taylor, J. Pene, and H. Yssel. 1998. IL-4 induces functional cell-surface expression of CXCR4 on human T cells. *J. Immunol. 160:4153.*
34. Ngo, V. N., L. H. Tang, and J. G. Cyster. 1998. Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells. *J. Exp. Med. 188:181.*
35. Willimann, K., D. F. Legler, M. Loetscher, R. S. Roos, M. B. Delgado, I. Clark-Lewis, M. Baggiolini, and B. Moser. 1998. The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7. *Eur. J. Immunol. 28:2025.*
36. Napolitano, M., A. Zingoni, G. Bernardini, G. Spinetti, A. Nista, C. T. Storlazzi, M. Rocchi, and A. Santoni. 1996. Molecular cloning of TER1, a chemokine receptor-like gene expressed by lymphoid tissues. *J. Immunol. 157:2759.*
37. Power, C. A., A. Meyer, K. Nemeth, K. B. Bacon, J. Hoogewerf, A. E. Proudfoot, and T. N. Wells. 1995. Molecular cloning and functional expression of a novel CC chemokine receptor cDNA from a human basophilic cell line. *J. Biol. Chem. 270:19495.*